Supplemental Tables

Dynamic Equilibrium between Multiple Active and Inactive Conformations Explains Regulation and Oncogenic Mutations in ErbB Receptors

Meytal Landau and Nir Ben-Tal

Table 1: Oncogenic mutations in EGFR and ErbB2, found in cancer cells and characterized in vitro

Mutation	Cited Report	Experimental Results	Functional and Structural Characterization	Notes
EGFR Alterations				
Missense Mutations in the EGFR				
E685X (E709X) X = [A,G,K,H]	Mutations in Glu685 were reported in lung cancer [1, 2]. E685H was found as a second mutation to Leu834 or to Gly695 [3].	Mutations in Glu685 increased the basal activity of the EGFR and its sensitivity to EGFR kinase inhibitors [4].	Glu685 is located on the interface of the active asymmetric dimer, and is predicted to stabilize a specific active dimeric form or change the native interactions between the activated and activating monomers within the dimer.	
G695X (G719X) X = (A, C, S, D)	Mutations in Gly695 were reported in lung cancer [2, 5-7].	Mutations in Gly695 increased the basal and EGF-induced activity of the EGFR, its oncogenic potential at the cellular level, and its sensitivity to EGFR kinase inhibitors [4, 8-11].	Gly695 is the first glycine within the GXGXXG motif in the phosphate-binding loop (P-loop). The mutation might exert its effect by directly influencing the phosphate transfer reaction via lowering the dissociation rates	Mutations in Gly695 represent 3%–4% of mutations in lung cancers [12, 13]. Of patient who sustained this mutation, 56%

		Mutations showed no evidence of impairing the maximal EGF response or altering the binding affinity for EGF [11]. We note that the effects were seen only with stable expressions of the mutant.	of ATP (and of ATP-analog inhibitors). Accordingly, G695S is more sensitive than the wt EGFR to ATP-analog EGFR kinase inhibitors [8, 10]. However, probably because the inhibitors display higher affinity for the active conformation, G695S is less sensitive to the inhibitors than the L834R and L837Q mutations that induce a ligand-independent active form.	responded to treatment with EGFR kinase inhibitor [13]. A mutation in the corresponding residue in the B-raf Ser/Thr kinase (Gly463) was detected in cancer cells and was oncogenic in vitro [14].
S744I (S768I)	S744I was reported in lung cancer [5, 15]. S744I was also reported as a second mutation to G719C/S [1].	Compared to the wt**,** S744I displayed increased basal kinase activity and greater sensitivity to EGFR kinase inhibitors such as gefitinib [4].	Ser744 is located at the C-terminus of the αC-helix. Because this position is close to the interface of the active dimer, the effect of the mutation may be related to alteration in the native orientation within active asymmetric hetero-dimers.	The corresponding residue in ErbB2 (Gly766) also undergoes substitutions in cancer cells [16], suggesting that these mutations are indeed oncogenic.
L834X (L858X) X = [R,M]	L834R was reported in lung cancer [1, 2, 5-7, 15, 17]. L834M was reported in lung cancer [2].	L834R increased basal and EGF-induced activity of the EGFR, its oncogenic potential at the cellular level, and its sensitivity to EGFR kinase inhibitors [4, 8-11]. L834R did not appear to impair the maximal EGF response. However, its affinity for EGF in the low-affinity binding mode was twofold greater than that of the wt [11].	L834R is located on the activation loop. It is predicted to cause destabilization of the inactive conformation of the kinase domain.	L834R accounts for 41%–43% of the mutations in lung cancers [12, 13]. Of the patient who sustained this mutation, 71% responded to treatment with EGFR kinase inhibitor [13]. A mutation in the corresponding

		We note that the effects were seen only with stable expressions of the mutants.		residue in the B-raf Ser/Thr kinase (Leu596) was detected in cancer cells and was oncogenic in vitro [14].
L837Q (L861Q)	L837Q was reported in lung cancer [1, 2, 6, 17].	L837Q increased the basal and EGF-induced activity of the EGFR, its oncogenic potential at the cellular level, and its sensitivity to EGFR kinase inhibitors [4, 11]. L837Q did not appear to impair the maximal EGF response [11].	L837Q is located on the activation loop. It is predicted to cause destabilization of the inactive conformation of the kinase domain.	The corresponding residue in ErbB2 (Leu869) also undergoes substitutions in cancer cells [18], suggesting that this mutations are indeed oncogenic. Also, mutations in the corresponding positions in the B-raf Ser/Thr kinase (Val599), the murine (D814V) [19] and human (D816V/H) [20] c-Kit tyrosine kinase, and the C-fms tyrosine kinase (D802V) [21] were detected in cancer cells and were oncogenic in-vitro [14, 19, 21].

In-Frame Deletions in *Exon19* in the EGFR				
ΔE722–A726 (ΔE746–A750)	Reported in lung cancer [1, 3, 5-7, 15].	The deletions increased the basal and EGF-induced activity of the EGFR, its oncogenic potential at the cellular level, and its sensitivity to EGFR kinase inhibitors [4, 9-11]. ΔL723-P729insS and ΔS728-I735 showed substantially reduced maximal levels of EGF-induced auto-phosphorylation [11]. We note that the effects were seen only with stable expressions of the mutants.	The deletions in *exon19* are located in the loop preceding the αC-helix and its N-terminus. They are predicted to cause destabilization of the inactive conformation of the kinase domain. However, the deletions would also compromise both the catalytically competent conformation of the kinase domain and the interactions within the active intracellular dimer, leading to reduced ligand-induced activity.	*Exon19* deletion accounts for 44%–48% of mutations in lung cancers [12, 13]. Of the patient sustaining these deletions, 84% responded to treatment with EGFR kinase inhibitor [13].
ΔL723–S728 (ΔL747–S752)	Reported in lung cancer [15].			
ΔL723–P729insS (ΔL747–P753insS)	Reported in lung cancer [6].			
ΔL723–E725_A726P (ΔL747–E749_A750P)	Reported in lung cancer [10].			
ΔS728–I735 (ΔS752–I759)	Reported in lung cancer [3, 7].			
In-Frame Insertions/Duplications in *Exon20* in the EGFR				
D746insNPG (D770insNPG)	Reported in lung cancer [4].	The insertion increased the basal activity of the EGFR. This mutant was remarkably insensitive to gefitinib and	The insertions in *exon20* are located in the loop following the αC-helix. This loop is located close to the interface of the symmetric,	Overall *exon20* insertions account for 3.7%–5% of the alterations in lung cancers [12, 13].

		erlotinib. Consistent with this result, all three lung adenocarcinoma patients with known *exon20* insertion mutants of EGFR failed to show a clinical response to treatment. However, the mutant was sensitive to treatment with an irreversible inhibitor, CL-387,785[10].	putatively inactive crystallographic dimer of the kinase domain, facing the equivalent loop from the second monomer. The insertions are predicted to cause destabilization of the inactive form. The mechanism causing insensitivity to the inhibitors is not yet clear.	
		ErbB2 Alterations		
		In-frame Insertions/Duplications in *Exon20* in ErbB2		
G776insYVMA (Ser744 in the EGFR).	Insertions following residue Gly776 were reported in cancer cells [5, 22-25].	G776insYVMA showed more potent autocatalytic activity than wt ErbB2 [25]. Heterodimers constituting the EGFR and the G776insYVMA ErbB2 mutant did not respond to ligand binding; moreover, they did not require contact formation between their extracellular domains [25].	These insertions are located within the binding site of Hsp90 [26], and close to the interface of the asymmetric active dimer of the kinase domain. Binding of Hsp90 restrains the activity of ErbB2, probably because of interference with formation of the asymmetric active dimer in which ErbB2 contacts the second monomer via its N-lobe. The alterations probably affect the active dimeric state. Specifically, they alter the native orientation of the monomers within the heterodimer	The most prevalent ErbB2 alterations found to date in cancer cells are insertions in *exon20*. These insertions were detected within residues 774–783 of ErbB2, which correspond to residues 742–751 of the EGFR [5, 16, 17, 22-24, 27].

			such that, in contrast to the wt dimer, ErbB2 is the activated monomer that phosphorylates the EGFR.	

Table 1: Alterations in EGFR are classified into three groups: missense mutations, *exon19* deletions, and *exon20* insertions. Alterations in ErbB2 comprise only an insertion in *exon20*. Positions of the mutations in the mature EGFR are indicated (numbering in pre-mature EGFR is in parenthesis). For the mutation in ErbB2, the corresponding residue in the mature EGFR is indicated in parenthesis.

Table 2: Mutations in EGFR, ErbB2, and ErbB4, detected in cancer cells but not yet characterized in-vitro.

Mutation	Cited Report	Functional and Structural Characterization	Predicted Effects
EGFR Alterations			
Missense Mutations in the EGFR			
G700S (G724S)	Reported in lung cancer [2].	Gly700 is located on the P-loop within the glycine-rich motif. It is conserved in ErbB and tyrosine kinases in general.	G700S is predicted to display a similar effect to that of the G695X mutation, which increases catalytic activity. The mutation might enhance catalysis. We note that the corresponding position in the B-Raf Ser/Thr kinase (G468A) was also found to be mutated in cancer cells [14].
E710K (E734K)	Reported in lung cancer [2].	Glu710 is located close to the interface within the active asymmetric dimer contributed by the N-lobe. This residue could be involved in a salt-bridge with the second monomer. This position is conserved as aspartate or glutamate residues in ErbBs from vertebrates.	E710K could have an effect on the active dimeric state.
L723F (L747F)	Reported in lung cancer [2].	Leu723 is located at the loop preceding the αC-helix, at the beginning of many *exon19* deletions found in cancer patients. It forms contacts with the activation loop in the inactive state and with the αC-helix in the active state.	The mutation might be involved in destabilization of the inactive state of the kinase domain, like the *exon19* deletions and other missense mutations in the αC-helix and activation loop. We note that the corresponding residue in

		Leu723 is conserved in ErbBs from vertebrates, except for ErbB3, which displays isoleucine in this position.	ErbB2 (Leu755) also undergoes substitutions in cancer, suggesting that these mutations are indeed oncogenic. The exceptional residue occupying this position in ErbB3 might point to a less stable inactive state of the kinase domain in ErbB3.
R724P (R748P)	Reported in lung cancer by [2].	Arg724 is located on the loop preceding the αC-helix. However, its side chain faces the solvent and does not participate in the inactive hydrophobic packing of the kinase domain. It is not conserved in the ErbBs, not even within the same ErbBs in different species.	Predicted to be polymorphic, according to the evolutionary conservation analysis and structural location.
V745X (V769X) X = [M, L]	V745L was reported together with S744I (S768I) on the same sequence [3]. V745M was reported together with an in-frame deletion in *exon19* on the same sequence [1].	Val745 is located at the end of the αC-helix, but does not participate in the packing with the activation loop in the inactive state. Conserved as a hydrophobic residue within the ErbBs, mostly as valine, leucine, and few methionine residues.	Because this residue is located in a regulatory element the mutation might be damaging, although the mild nature of the substations weakens this prediction. Nevertheless, the fact that the corresponding residue in ErbB2 (Val777) undergoes similar substitutions in cancer cells points to the oncogenic nature of these mutations.
H749R (H773R)	H749R was reported together with W731Stop in patients who did not respond to EGFR kinase inhibitors. [1].	The position corresponding to H749R in ErbBs from vertebrates is occupied by histidine or tyrosine. It is also conserved in tyrosine kinases as histidine, tyrosine, or asparagine residues. It is substituted for arginine in an oncogenic	Because of its conservation pattern in tyrosine kinases and ErbBs, and its close proximity to an essential residue, this mutation is predicted to be damaging. However, the nature of the effect is not yet known.

		viral EGFR variant. His749 is located spatially close to Lys799, which was shown to be essential for EGFR function [28].	
R752C (R776C)	R752C was reported as a second mutation to L834 or G695X [3, 15].	Arg752 is located on the interface of the symmetric, putatively inactive dimer. It is conserved in ErbBs from vertebrates.	R752C might participate in destabilization of the inactive dimeric conformation, leading to heightened basal activity. We note that the corresponding residue in ErbB4 also undergoes substitution in cancer cells, suggesting that these mutations are indeed oncogenic.
Q763R (Q787R)	Reported in lung cancer [2].	Gln763 is conserved in ErbBs from vertebrates and is buried within the protein in both the active and the inactive conformations.	Because of its conservation pattern in ErbBs and its structural location, Q763R is predicted to be damaging. However, the nature of the effect is not yet known.
T766M (T790M)	Reported in lung cancer [2].	Thr766 is located in the ATP-binding pocket, and contacts the ATP-analog in the crystal structure [29]. It is conserved in ErbBs from vertebrates.	T766M is responsible for at least half of the acquired resistance to EGFR kinase inhibitors such as gefitinib and erlotinib. Substitution of a bulkier residue, such as methionine, for threonine is thought to sterically hinder the binding of these drugs (reviewed in [13]). A mutation in the corresponding residue in the ABL1 tyrosine kinase (T315I) is also related to an acquired resistance in ABL1, (reviewed in [13]).

L809V (L833V)	L809V was reported in lung cancer [2]. L809V was also reported as a second mutation to H811L [1].	Leu809 is located close to the activation loop and αC-helix in the inactive conformation, and contacts Phe832 from the conserved DFG motif [30]. Leu809 is conserved as a leucine residue in all ErbBs except ErbB3, which displays methionine in this position. A methionine in this position is also displayed by a viral variant of the EGFR.	L809V might participate in destabilization of the hydrophobic packing in the inactive conformation of the kinase domain. The exceptional residue occupying this position in ErbB3 might point to a less stable inactive state of the kinase domain in ErbB3.
V810L (V834L)	V810L was reported in lung cancer [2].	Val810 interacts with residues from the conserved catalytic loop (residues 811−813). In the inactive conformation it is packed directly against Arg812. Val810 is largely conserved in ErbBs.	The mutation might stabilize the active state of the catalytic loop in the kinase domain. We note that the corresponding residue in ErbB2 (Val842) also undergoes substitution in cancer cells, suggesting that these mutations are indeed oncogenic.
H811L (H835L)	H811L was reported in lung cancer [15].	His811 is a part of the conserved catalytic loop (residues 811−813; Asp813 is the proton acceptor catalytic residue). His811 is totally conserved in ErbBs and tyrosine kinases.	The evolutionary conservation and location of His811 suggest that mutation in this position would be damaging. However, the nature of the effect is not yet known.
L814V (L838V)	L814V was reported in lung cancer as a second mutation to L834R [1].	Leu814 follows the conserved catalytic loop (residues 811−813). Leu814 is conserved in ErbBs and is completely buried within the protein.	Because of the conservation pattern and structural location of this residue, the mutation is predicted to be damaging. However, the nature of the effect is not yet known.
A815T (A839T)	A815T was reported in lung cancer [1].	Ala815 follows the conserved catalytic loop (residues 811−813).	Because of the conservation pattern and structural location of this residue, the mutation is

		Ala815 is conserved in ErbBs and is completely buried within the protein.	predicted to be damaging. However, the nature of the effect is not yet known.
K822R (K846R)	K822R was reported in lung cancer [1].	Lys822 is located on the interface of the crystallographic symmetric dimer involved in the polar network within the complex. Lys822 is conserved in ErbBs from vertebrates.	K822R might lead to destabilization of the inactive dimer.
G849E (G873E)	G849E was reported in lung cancer [2].	Gly849 is located on the activation loop. Gly849 is conserved in ErbBs from vertebrates, except for ErbB3, which displays a glutamate residue in this position (as in the mutant).	G849E might lead to destabilization of the inactive state of the kinase domain. Glutamate was previously shown to mimic a phosphate in the activation loop, leading to activation.
In-Frame Deletions in *Exon19* in the EGFR			
Δ722–727 insA (Δ746–751insA)	Reported with V769M [1].	The *exon19* deletions are located in the loop preceding the αC-helix and in its N-terminus.	*Exon19* deletion accounts for 44%–48% of mutations in lung cancers [12, 13]. Of the patient sustaining these deletions, 84% respond to treatment with EGFR kinase inhibitors [13]. These deletions are predicted to have a similar effect to those tested in vitro (such as ΔL723-P729insS), which show increased basal activity and enhanced transforming potential [11], probably because of destabilization of the inactive conformation of the kinase domain.
Δ722–727 insI (Δ746–751insI)	Reported by [15].		
Δ722–728 insV (Δ746–752insV)	Reported by [5].		
Δ722–728 insD (Δ746–752insD)	Reported by [1].		
Δ723–726insP (Δ747–750insP)	Reported by [3].		
Δ723–727 (Δ747–751)	Reported by [1, 3].		
Δ723–727insS (Δ747–751insS)	Reported by [6].		
Δ723–728 (Δ747–752)	Reported by [3, 15].		
Δ723–728 insQ (Δ747–752insQ)	Reported by [15].		

Δ723–729insS (Δ747–753insS)	Reported by [6].		
720insKIPVAI (744insKIPVAI)	Reported by [3].	Located on a β-strand in the N-lobe.	This is an unexpected insertion in *exon19*. The effect of this mutation could not be predicted.
In-Frame Insertions/Duplications in *Exon20* in the EGFR			
D737insEAFQ (D761insEAFQ)	Reported by [1, 3].	The *exon20* insertions are located in the C-terminus of the αC-helix and its following loop.	*Exon20* insertions constitute 3.7%–5% of alterations in lung cancers [12, 13]. *Exon20* insertions, like the wt EGFR, are sensitive to EGFR kinase inhibitors [13]. All of these insertion are predicted to show increased basal activity and enhanced transforming potential, similar to the D746insNPG alteration tested in vitro [10]. Nevertheless, the molecular effects of these insertions might differ, depending on their exact location. For example, insertions C-terminal to residue 746 are predicted to interfere with the interface of the symmetric, putatively inactive dimer. On the other hand, insertions located at the C-terminus of the αC-helix might participate in destabilization of the inactive state of the kinase domain. Alternatively, because the αC-helix constitutes the interface of the active dimer, these insertions might have an effect dimer formation.
A743insTLA (A767insTLA)	Reported by [3].		
Dup744-746 (Dup768–770)	Reported by [5].		
V745insASV (769insASV)	Reported by [3].		
D746insNPG (D770insNPG)	Reported by [4].		
D746GinsY (D770GinsY)	Reported by [3].		
Dup747–749 (Dup771–773)	Reported by [5].		

ErbB2 Alterations			
Missence Mutations in ErbB2			
K724N (Lys692 in the EGFR)	K724N was reported in gastric cancers [18].	A model of ErbB2, based on the EGFR, predicted that Lys724 is exposed to the surface and is located close to the interface of the symmetric putatively inactive dimer. Lys724 is conserved in ErbBs from vertebrates.	Lys724 might be important in the electrostatic complementarity between the kinase and the C-terminal domains within the symmetric dimer. Therefore, the mutation might lead to destabilization of the inactive dimeric state.
T733I (Thr701 in the EGFR)	T733I was reported in gastric cancers [18].	Thr733 is located within the glycine-rich motif on the P-loop. It is conserved in ErbBs from vertebrates.	T733I might affect the phosphate transfer reaction.
L755X X= [P, S] (Leu723 in the EGFR)	L755P was reported in lung cancer [5, 16, 24]. L755S was reported in gastric and breast cancers [18].	A model of ErbB2, based on the EGFR, predicted that Leu755 is located at the loop preceding the αC-helix. Leu755 is conserved in ErbBs from vertebrates, except for ErbB3, which displays isoleucine in this position.	The corresponding position to ErbB2's L755 in the EGFR is L723, which was also detected in cancer cells. (See above for analysis of this position.)
D769H (Asp737 in the EGFR)	D769H was reported in lung and gastric cancers [18, 31].	A model of ErbB2, based on the EGFR, predicted that Asp769 is located at the αC-helix, on the interface of the asymmetric dimer. The residue faces toward the solvent, not toward the hydrophobic packing with the activation loop. Asp769 is conserved in ErbBs from vertebrates.	D769H is predicted to affect formation of the active dimer.
V773A (Val741 in the EGFR)	V773A was reported in carcinoma of the head and neck [32].	A model of ErbB2, based on the EGFR, predicted that Val773 is located in the αC-helix, facing toward the protein. It is buried in the active	Because of its location on a regulatory element and its unique pattern of substitution, we predict that Val773 is functionally important and that its

		conformation, contacting a conserved motif at the N-terminus of the activation loop. Val773 is conserved as a valine residue in the EGFR and ErbB2 and as an isoleucine residue in ErbB4. In ErbB3 the corresponding position is mostly occupied by alanine (or serine in two fish orthologs).	substitution could be damaging. However, the nature of the effect of the substitution is not yet known.
G776S (Ser744 in the EGFR)	G776S was reported in gastric tumors [16].	A model of ErbB2, based on the EGFR, predicted that Gly776 is located at the C-terminus of the αC-helix.	The position corresponding to ErbB2's Gly766 in the EGFR is Ser744, which was also found to be mutated in cancer cells and was shown to increase the basal activity of the receptor. (See Table1 for analysis of this position.)
V777X X= [L, M] (Val745 in the EGFR)	V777L was reported in lung, colorectal and gastric cancers [18, 24]. V777M was reported in colorectal cancers [18].	A model of ErbB2, based on the EGFR, predicted that Val777 is located at the end of the αC-helix.	The position corresponding to ErbB2's Val777 in the EGFR is Val745, which was also detected in cancer cells. (See above for analysis of this position.)
Q799P (Gln767 in the EGFR)	Q799P was reported in gastric cancer [18].	A model of ErbB2, based on the EGFR, predicted that Gln799 is located on the interface of the symmetric putatively inactive dimer, contacts the C-terminal domain, and participates in the polar network of interactions across the dimer. Gln799 is conserved in ErbBs as a glutamine residue.	Q799P is predicted to lead to destabilization of the inactive dimeric state.

V842I (Val810 in the EGFR)	V842I was reported in colorectal cancer [18].	A model of ErbB2, based on the EGFR, predicted that the location of Val842 is close to the conserved catalytic loop.	The position corresponding to ErbB2's Val842 in the EGFR is Val810, which was also detected in cancer cells. (See above for the analysis of this position.)
N857S (Gln825 in the EGFR).	N857S was reported in an ovarian tumor [16].	A model of ErbB2, based on the EGFR, predicted that Asn857 is located on a loop on the back side of the catalytic site and is exposed to the solvent. Asn857 is not conserved in ErbBs.	Based on the evolutionary conservation analysis and its structural location, N857S is predicted to be a neutral mutation.
L869Q (Leu837 in the EGFR)	L869Q was reported in gastric cancer [18].	A model of ErbB2, based on the EGFR, predicted that Leu869 is located on the activation loop.	The position corresponding to ErbB2's Leu869 in the EGFR is Leu837, which was also detected in cancer cells and was shown to increase the basal activity of the receptor. (See above for the analysis.)
R896C (His864 in the EGFR)	R896C Was reported in breast cancer [18].	A model of ErbB2, based on the EGFR, predicted that Arg896 is located on a loop and is exposed to the surface. This residue is not conserved in ErbBs, but is evolutionarily variable.	Based on the conservation analysis and its structural location, this mutation is predicted to be neutral in its effect.
E914K (Glu882 in the EGFR)	E914K was reported in glioblastoma [16].	A model of ErbB2, based on the EGFR, predicted that Glu914 is located on a helix in the C-lobe. It is relatively buried in a hydrophobic environment, yet participates in forming an H-bond with the nitrogen of W856. Glu914 is conserved in ErbBs.	Based on the evolutionary conservation analysis this mutation is predicted to be damaging; however, the nature of the effect is not yet known.

In-frame Insertions/Duplications in *Exon20* in ErbB2			
774insAYVM (742 in the EGFR)	Reported in lung cancer [16, 23].	The insertions are located on the C-terminus of the αC-helix and its following loop and are close to the interface of the symmetric dimer.	All of these insertion are predicted to increase the basal activity and enhance transforming potential, similar to the G776insYVMA ErbB2 mutant [25] and the D746insNPG EGFR mutant [10] tested in vitro. Nevertheless, the molecular effects of these insertions might vary. The loop following the αC-helix in ErbB2 is known to bind Hsp90, which plays a role in restraining the activity of ErbB2, probably due to interference with formation of the asymmetric active dimer in which ErbB2 contacts the second monomer va its N-lobe. The alterations might have an effect on the active dimeric state.On the other hand, the two insertions that are located following residue 778 in ErbB2 (746 in the EGFR) might participate in destabilization of the inactive dimeric state.
775insYVMA (743 in EGFR)	Reported in lung cancer [17].		
G776XinsC (744 in EGFR) X = [L, V]	G776VinsC was reported in lung cancer [5]. G776LinsC was reported in lung cancer [22, 23].		
776-779insYVMA (S744–N747 in EGFR)	Reported in lung cancer [22, 24].		
779-781ins VGS (747–749 in EGFR)	Reported in lung cancer [16].		
781–783insGSP (749–751 in EGFR)	Reported in lung cancer [22].		

ErbB4 Alterations			
Missense Mutations			
V721I (Ile691 in the EGFR)	Reported by [33].	In a model of ErbB4 based on the EGFR, Val721 is located on the interface of the symmetric, putatively inactive dimer, close to Asp1012 (Glu981 in the EGFR) from the C-terminal domain. Val721 is conserved as a hydrophobic residue in ErbBs from vertebrates. It is substituted for an aspartate residue in two oncogenic EGFRs from southern platyfish *(Xiphophorus maculates)* and spiketail platyfish *(Xiphophorus xiphidium)*.	This mutation is predicted to lead to destabilization of the inactive dimeric state, although this prediction is weakened by the mild nature of the mutations.
A773S (Ala743 in the EGFR)	Reported by [33].	In a model of ErbB4 based on the EGFR, Ala773 is located on the αC-helix, on the interface of the asymmetric active dimer. It faces outward, contacting Ile949 (Ile917 in the EGFR) from the second monomer. Ala773 is conserved in all ErbBs except for ErbB3 orthologs that contain a glycine residue.	It is predicted to affect the formation of the active dimeric state. The exceptional residue occupying this position in ErbB3 might be related to the fact that ErbB3 probably does not form asymmetric heterodimers via its N-lobe (thereby acting as the activated monomer). Therefore, there is no evolutionary constraint in this position within ErbB3 orthologs, as opposed to the catalytically active ErbBs. This further indicates that the mutation in this residue in ErbB4 might have an effect on functionality.

R782Q (Arg752 in the EGFR)	Reported by [33].	In a model of ErbB4 based on the EGFR, Arg782 is located on the interface of the symmetric, putatively inactive dimer.	The position corresponding to ErbB4's Arg782 in the EGFR is Arg752, which was also detected in cancer cells. (See above for analysis of this position.)
E810K (Glu780 in the EGFR)	Reported by [33].	In a model of ErbB4 based on the EGFR, Glu810 is located on the surface of the protein, where it is exposed to the solvent. Glu810 is conserved in ErbBs from vertebrates, except for ErbB3, which displays a glutamine residue in this position. This position is also substituted for glutamine in oncogenic EGFRs from southern platyfish (*X. maculatus*) and spiketail platyfish (*X. xiphidium*).	Because of the unique conservation pattern of this position in the ErbBs, we predict that the mutation is damaging and will lead to enhanced activation.
P854Q (Pro824 in the EGFR)	Reported by [33].	In a model of ErbB4 based on the EGFR, Pro854 is located on the surface of the kinase domain; close to the interface with the C-terminal domain. The proline residue causes a turn in the structure between two β-strands. Pro854 is conserved in all ErbBs from vertebrates, except for ErbB3 from fish, which contain an aspartate residue in this position.	Because of the unique conservation pattern of this position in the ErbBs and its structural location, we predict that the mutation is damaging and will lead to enhanced activation.
D861Y (Asp831 in the EGFR)	Reported by [33].	Asp861 is part of the conserved DFG motif [30] at the N-terminus of the activation loop and is crucial for catalysis. Asp861 is conserved in ErbBs and in tyrosine	Because of the pattern of conservation and the structural location of this residue, the mutation is predicted to be damaging. However, the nature of the effect is not yet known.

		kinases in general.	
E872K (Glu842 in the EGFR)	Reported by [33].	In a model of ErbB4 based on the EGFR, Glu872 is located on the activation loop. Glu872 is conserved as a glutamate or aspartate residue in ErbBs from vertebrates.	Because of the pattern of conservation and the structural location of this residue, we predict that the mutation is damaging. However, the nature of the effect is not yet known.
T926M (Ala896 in the EGFR)	Reported by [33].	In a model of ErbB4 based on the EGFR, Thr926 is located on the surface of the protein, and is exposed to the solvent. Thr926 is not conserved in ErbBs.	Based on the conservation analysis and the structural location of this residue, the mutation is predicted to be neutral.
In-Frame Insertions/Duplications in *Exon20* in ErbB4			
G802insGGC (Gly772 in the EGFR)	Reported by [33].	In a model of ErbB4 based on the EGFR, Gly802 is located on a strand preceding the first helix in the C-lobe, in contact with the ATP analog [29]. Gly802 is conserved in ErbBs and is also largely conserved in tyrosine kinases in general.	Gly802 might be important for ligand binding and for catalysis. The insertion is predicted to be damaging, but the nature of the effect is not yet known.

Table 2: Alterations in EGFR are classified into three groups: missense mutations, *exon19* deletions, and *exon20* insertions. Alterations in ErbB2 and ErbB4 include missense mutations and *exon20* insertions. For alterations in EGFR, positions of the mutations in the mature EGFR are indicated (numbering in pre-mature EGFR is in parenthesis). For mutations in ErbB2 and ErbB4, the corresponding residue in the mature EGFR is indicated in parenthesis.

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